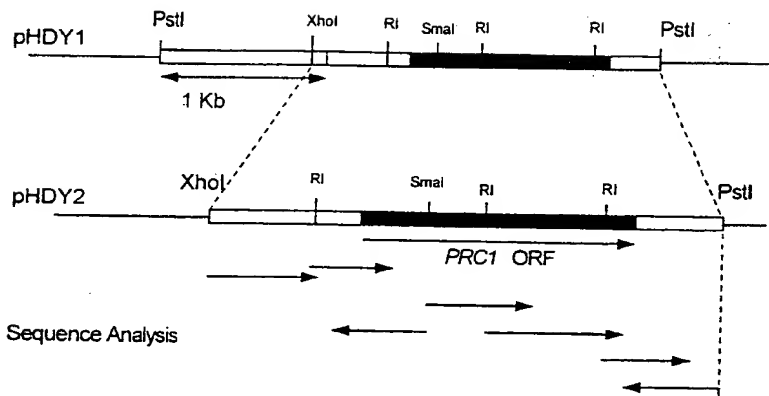
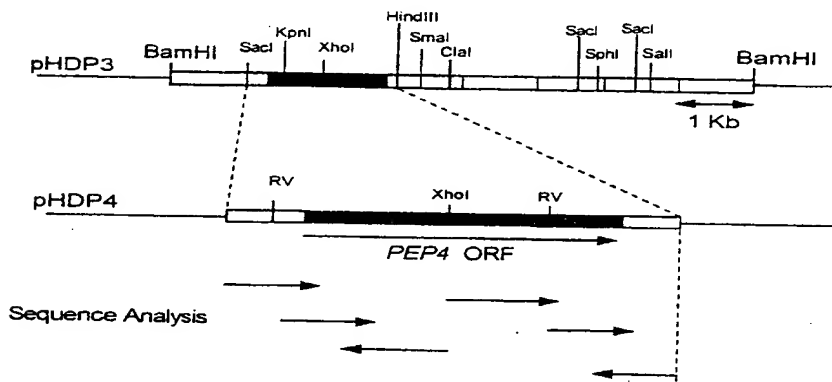
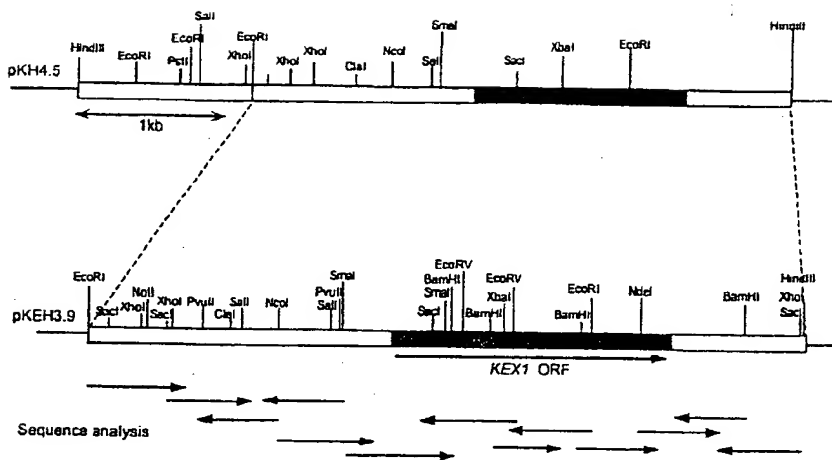


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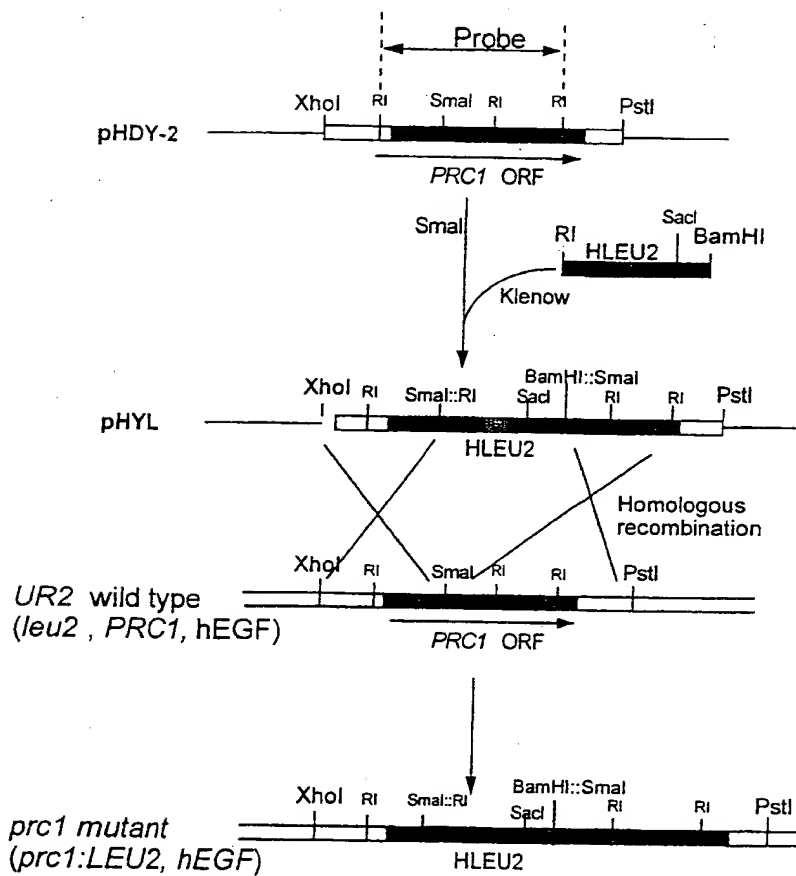
**FIG. 1****FIG. 2**

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**FIG. 3**

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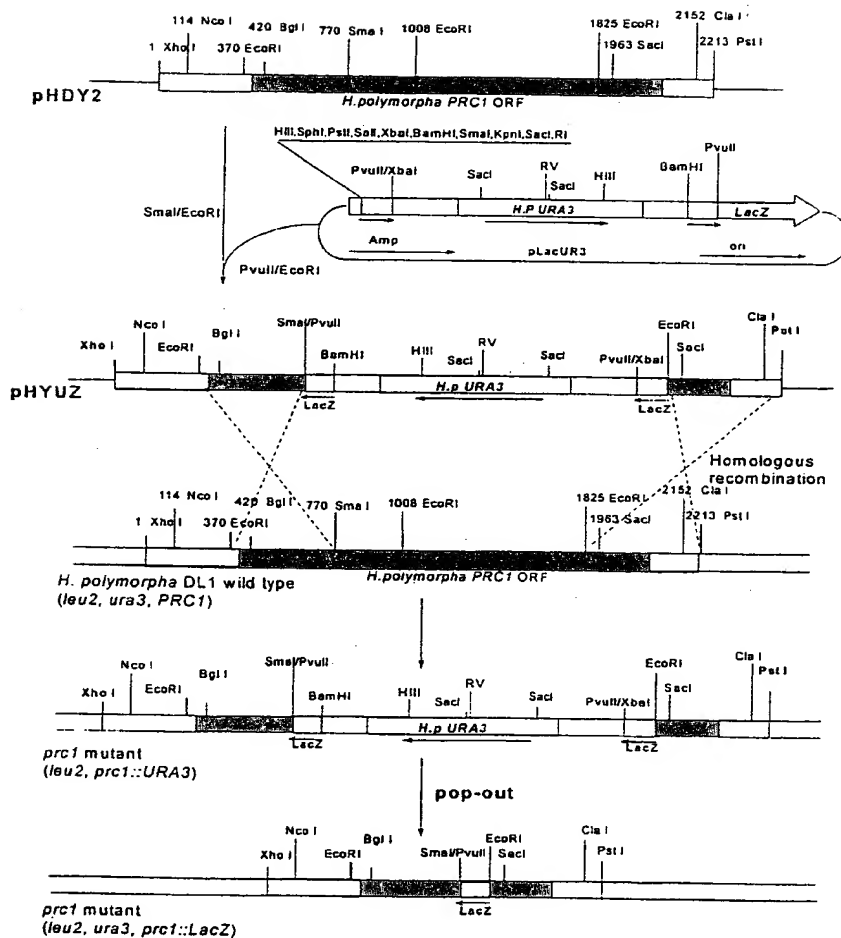
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**FIG. 4**

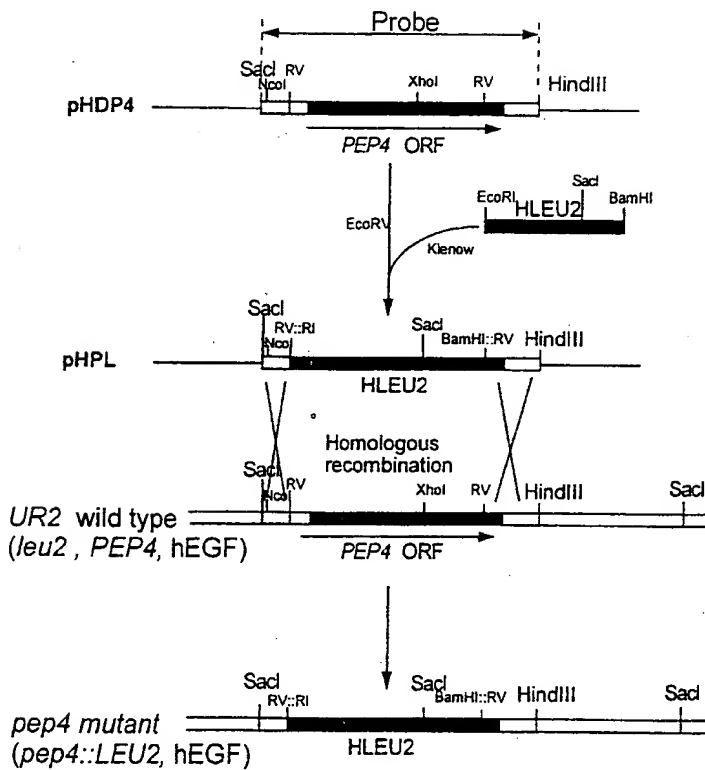
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FIG. 5



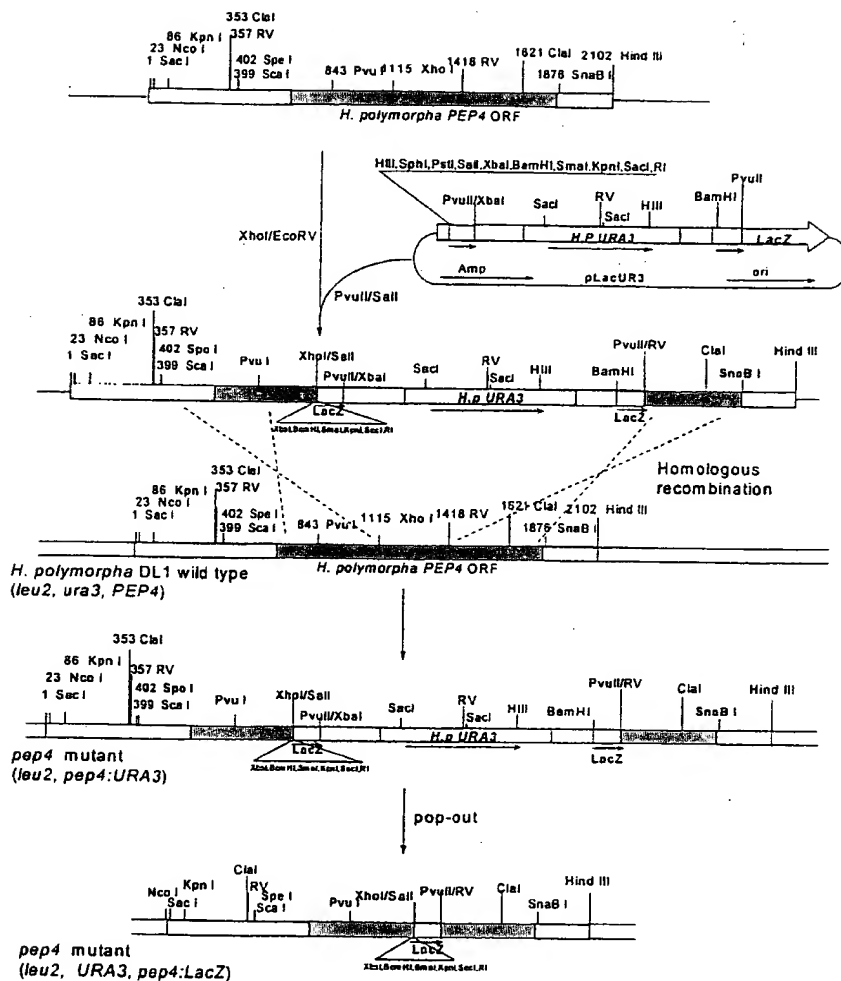
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**FIG. 6**

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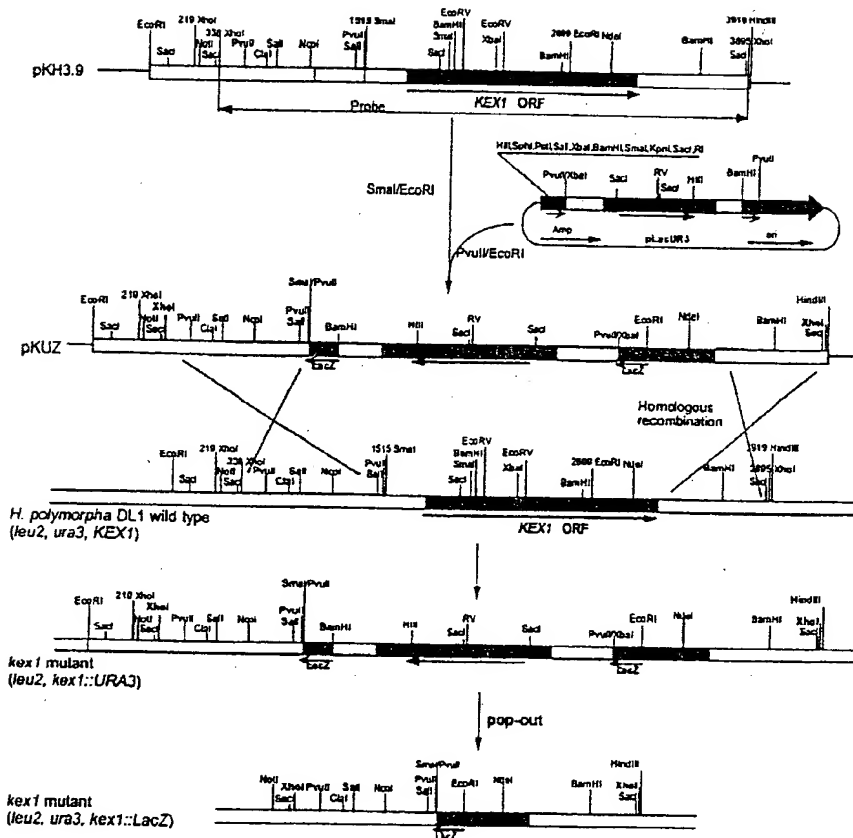
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FIG. 7



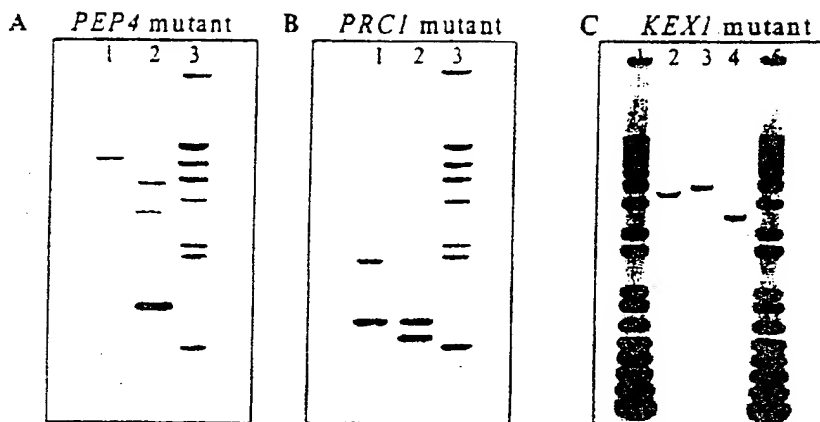
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FIG. 8



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## FIG. 9



A. Lane 1 : *H. polymorpha* UR2/*EcoRV*  
 Lane 2 : *pep4* mutant/*EcoRV*  
 Lane 3 : DIG- $\lambda$ /*Hind*III size marker  
 probe : DIG labeled pHDP4/*Sac*I/*Hind*III 2kb fragment

B. Lane 1 : *H. polymorpha* UR2/*EcoRI*  
 Lane 2 : *prc1* mutant/*EcoRI*  
 Lane 3 : DIG- $\lambda$ /*Hind*III size marker  
 probe : DIG labeled pHDY2/*EcoRI* 1.5kb fragment

C. Lane 1,5 :DIG-1 kb size marker  
 Lane 2 : *H. polymorpha* DL1/*Xho*I  
 Lane 3 : *kex1* mutant/*Xho*I  
 Lane 4 : URA3 gene pop-out/*Xho*I  
 probe : DIG labeled pKH4.5/*Xho*I 3.5kb fragment

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FIG. 10

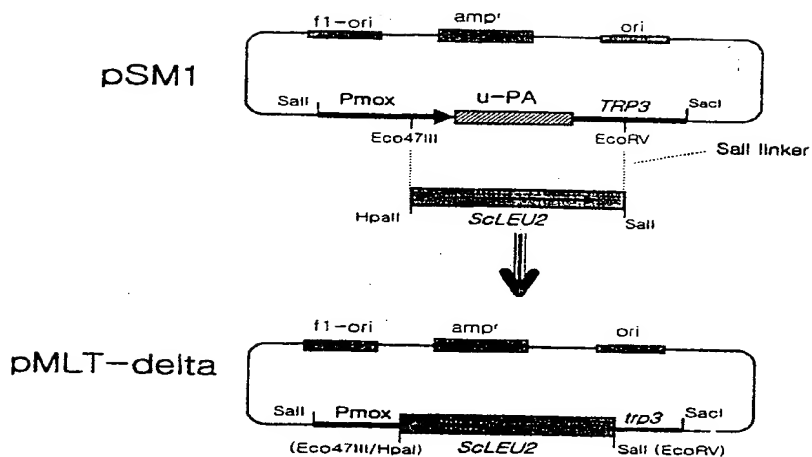
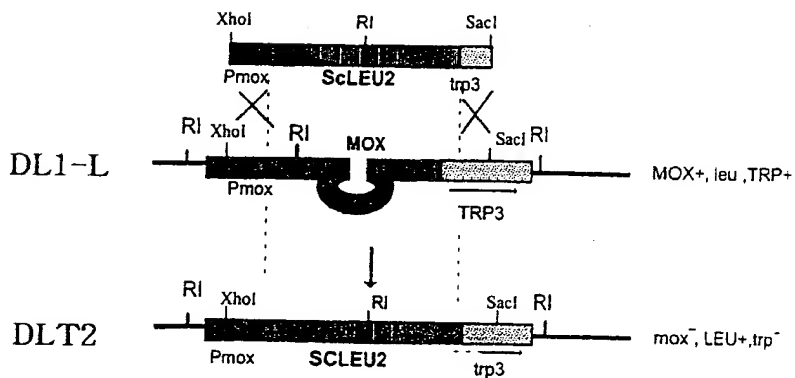


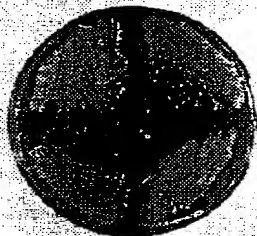
FIG. 11



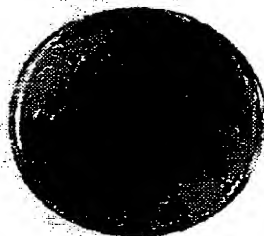
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**FIG. 12**

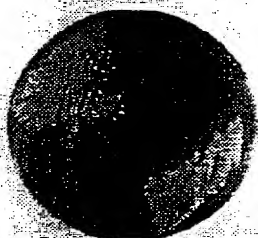
A. 2% Glucose + 0.67% YNB  
+ LEU + TRP



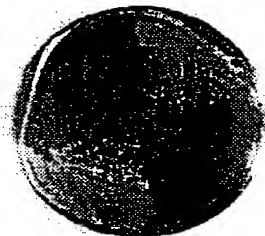
B. 0.5% Methanol + 0.67% YNB  
+ LEU + TRP



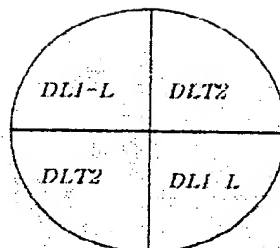
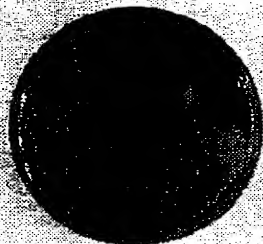
C. 2% Glucose + 0.67% YNB  
+ LEU



D. 2% Glucose + 0.67% YNB  
+ TRP

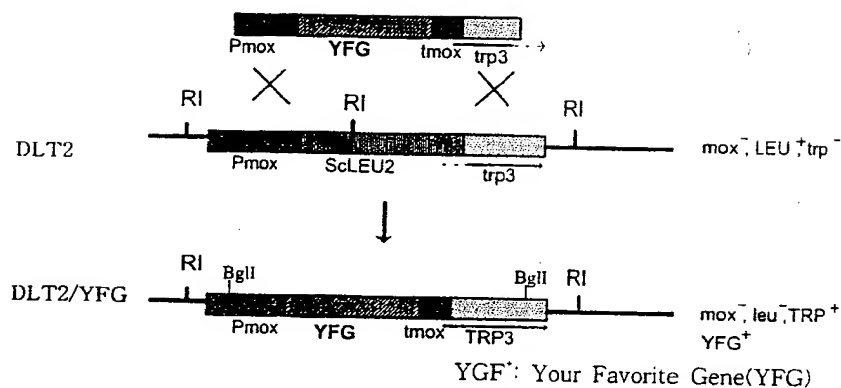
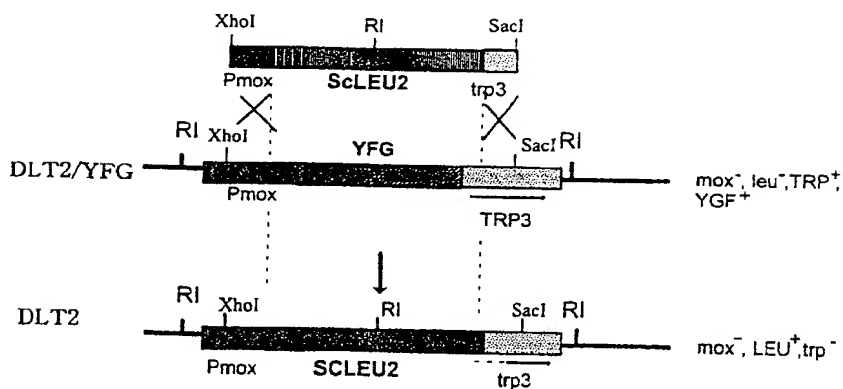


E. 2% Glucose + 2% Peptone  
+ 1% Yeast extract



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**FIG. 13a****FIG. 13b**

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FIG. 13c

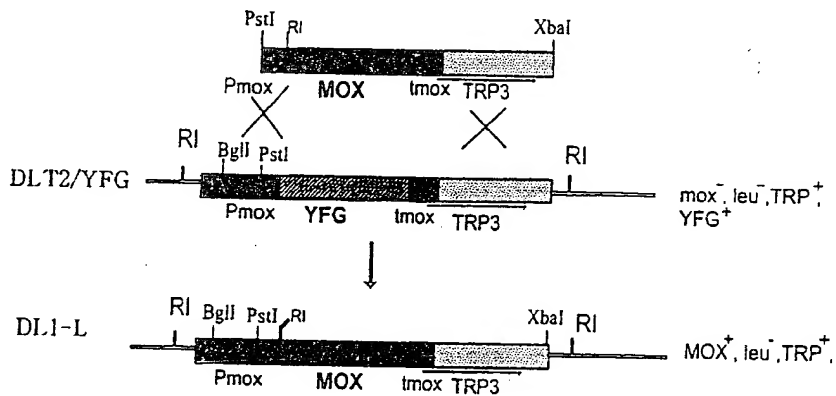
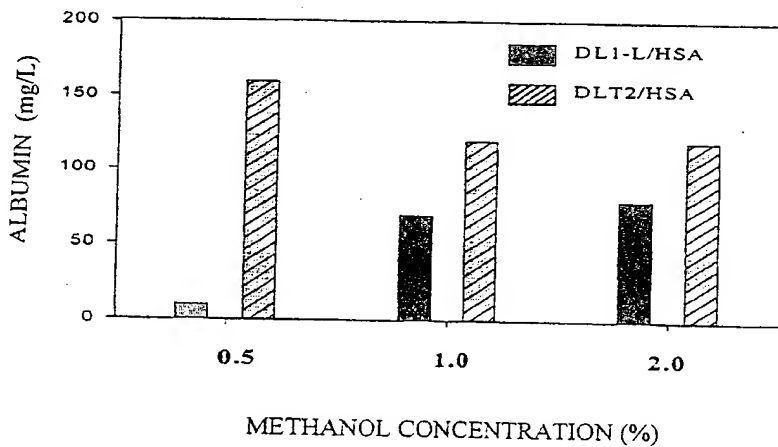


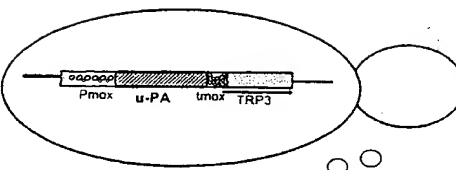
FIG. 14



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FIG. 15

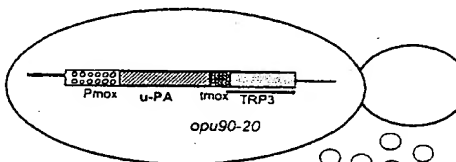
DLT2/u-PA

*(leu2 mox-trp3::u-PA)*

Selection of supersecretion mutant  
of u-PA after EMS mutagenesis

secretion of u-PA

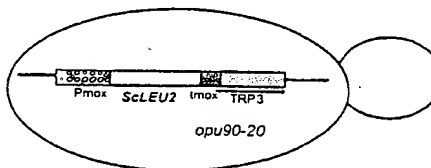
DLT90-20/u-PA

*(leu2 mox-trp3::u-PA)*

Pop-out of the u-PA expression cassette  
from the obtained supersecretion mutant  
DLT90-20/u-PA

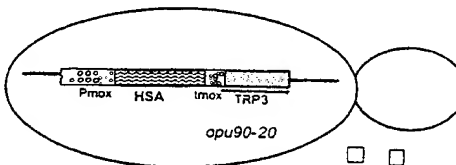
supersecretion of u-PA

DLT90-20

*(leu2 mox-trp3::ScLEU2)*

Introduction of the HSA expression  
cassette into the supersecretion  
mutant DLT90-20

DLT90-20/HSA

*(leu2 mox-trp3::HSA)*

supersecretion of HSA

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